CLAIMS

We claim:

1. A method of identifying a multifunctional oligomeric compound to modulate expression of RNA comprising:

(a) contacting a target RNA with one or more double-stranded oligomeric compounds hybridizable to one or more target regions of said RNA and identifying double-stranded oligomeric compounds which inhibit target RNA levels by at least 50%;

(b) contacting the target RNA with an antisense strand of said modulating double-stranded oligomeric compound and determining whether the antisense strand inhibits target RNA levels by at least 50%; and

(c) identifying said inhibiting antisense strand and said inhibiting double-stranded oligomeric compound as multifunctional oligomeric compounds.

2. A multifunctional oligomeric compound identified according to claim 1.

3. A method of claim 1 wherein the multifunctional oligomeric compound inhibits target RNA levels by at least 80%.

4. The method of claim 1 wherein the target region is identified by a single-stranded oligomeric gene walk across the target RNA.

5. The method of claim 1 wherein the target region is identified by secondary structure analysis of the target RNA.

6. The method of claim 1 wherein said target region is at least a portion of an induced gene.

7. The method of claim 6 wherein the induced gene is CD54.

8. The method of claim 1 wherein said target region is at least a portion of a constitutive gene.

9. The method of claim 1 wherein said target region is localized to the 3'UTR, the 5'UTR, an intron:exon boundary, an exon:exon boundary, a start region or a coding region of the RNA.

10. The method of claim 1 wherein said target region is localized to the 3'UTR.

11. The method of claim 1 wherein said target region is localized to the 5'UTR.

12. The method of claim 1 wherein said target region is localized to an intronic portion of a gene.

13. The method of claim 1 wherein said target region is localized to an exon.

14. The method of claim 1 wherein said target region is localized to an intron/exon boundary.

15. The method of claim 1 wherein said target regions overlaps the intron/exon boundary with 5-10 nucleotides on either side of the boundary.

16. A method for optimizing target region selection for modulation of RNA expression comprising:

(a) contacting one or more double-stranded oligomeric compounds with one or more regions of a target RNA and identifying target regions which, when contacted with the one or more double-stranded oligomeric compounds, result in inhibition of target RNA levels of at least 50%;

(b) contacting one or more single-stranded oligomeric compounds with said inhibited target regions and identifying regions which, when contacted with the one or more single-stranded oligomeric compounds, result in inhibition of target RNA levels of at least 50%;

(c) identifying regions modulated by at least one double-stranded oligomeric compound and at least one single-stranded oligomeric compound as optimized target regions.

17. The method of claim 16 wherein target RNA levels are inhibited by at least 80% by single-stranded oligomeric compounds and double-stranded oligomeric compounds.

18. The method of claim 1 wherein the oligomeric compound is an antisense oligonucleotide.

19. The method of claim 1 wherein the oligomeric compound has at least one modification of the base, sugar or internucleoside linkage.

20. The method of claim 1 wherein the oligomeric compound has a modification at the 2' position of at least one sugar.

21. The method of claim 1 wherein said oligomeric compound comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside.

22. The method of claim 1 wherein said oligomeric compound is from about 12 to about 50 nucleotides in length.

23. The method of claim 1 wherein said oligomeric compound is from about 18 to about 25 nucleotides in length.

24. The method of claim 1 wherein said oligomeric compound comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside; said modified nucleoside adapted to modulate at least one of; binding affinity or binding specificity of said oligomeric compound.

25. The method of claim 1 wherein the oligomeric compound is RNA.

26. The method of claim 1 wherein the oligomeric compound is a siRNA

27. The method of claim 1 wherein said hybridization is under moderate or high stringency conditions.

28. The method of claim 1 wherein the oligomeric compound is a potent modulator of the target RNA.

29. The method of claim 1 wherein the oligomeric compound is a gapmer.

30. The method of claim 1 wherein the oligomeric compound comprises at least six consecutive nucleosides with 2' modifications.

31. The method of claim 1 wherein the oligomeric compound is a hemimer.

32. The method of claim 1 wherein the oligomeric compound comprises at least one phosphorothioate linkage.

33. The method of claim 1 wherein the oligomeric compound is a chimeric compound.

34. The method of claim 1 wherein the oligomeric compound comprises one or more chimeric regions.

35. The method of claim 1 wherein the target RNA is preselected.

36. A method of modulating RNA expression comprising contacting target regions optimized according to claim 16 with two or more oligomeric compounds.

37. A method of optimizing modulation of RNA comprising contacting a target RNA with at least two oligomeric compounds hybridizable to a target region of said target RNA wherein at least two oligomeric compounds each inhibit RNA levels by at least 50% when tested individually.

38. A method of optimizing target regions of RNA comprising:

(a) contacting a target RNA comprising a target region with a plurality of oligomeric compounds hybridizable with said target region; and,

(b) identifying target regions as optimized when two or more of said oligomeric compounds inhibit target RNA levels by at least 50%.

39. The method of claim 38 wherein the oligomeric compound comprises at least one double-stranded region.

40. The method of claim 38 wherein target regions are identified as optimized when two or more of said oligomeric compounds inhibit target RNA levels by at least 80%.

41. A method of selecting a target region of a gene comprising:

(a) contacting a target RNA comprising at least one target region with a plurality of oligomeric compounds hybridizable with said at least one target region, wherein said oligomeric compounds comprise at least one siRNA oligomeric compound and at least one ASO oligomeric compound;

(b) identifying siRNA and ASO oligomeric compounds which inhibit RNA levels by at least 60% for each of said at least one target regions; and

(c) selecting target regions when there is a significant association between inhibiting siRNA oligomeric compounds and ASO oligomeric compounds for the target region.

42. The method of claim 41 wherein at least one of said oligomeric compounds comprises at least one double-stranded region.

43. A method of claim 41 wherein (c) is performed using a ROC analysis.

44. A method of claim 43 wherein the ROC analysis yields an area under the curve of at least 0.6 .

45. A method of claim 43 wherein the ROC analysis yields an area under the curve of at least 0.8 .

46. A target region of a gene selected according to the method of claim 41.

47. A method of selecting an optimized single-stranded oligomeric compound comprising:

(a) contacting a target RNA with one or more double-stranded oligomeric compounds;

(b) identifying one or more double-stranded oligomeric compounds which inhibit target RNA levels by at least 50%; and

(c) selecting the strand of the double-stranded oligomeric compound that hybridizes to the target RNA as the optimized single-stranded oligomeric compound.

48. The method of claim 47 wherein target RNA levels are inhibited by at least 80%.

49. A method of selecting an optimized double-stranded oligomeric compound comprising:

(a) contacting a target RNA with one or more single-stranded oligomeric compounds;

(b) identifying one or more single-stranded oligomeric compounds which inhibit target RNA levels by at least 50%; and

(c) hybridizing a complementary single-stranded oligomeric compound to said single-stranded oligomeric compound, thereby yielding an optimized double-stranded oligomeric compound.

50. A method of selecting a single-stranded oligomeric compound comprising;

(a) contacting a target RNA with one or more double-stranded oligomeric compounds;

(b) identifying one or more double-stranded oligomeric compounds which inhibit target RNA levels by at least 50%; and

(c) selecting the strand of the identified double-stranded oligomeric compound which is complementary to the target RNA as the selected single-stranded oligomeric compound.

51. A method of selecting a double-stranded oligomeric compound comprising:

(a) contacting a target RNA with one or more single-stranded oligomeric compounds;

(b) identifying one or more single-stranded oligomeric compounds which inhibit target RNA levels by at least 50%; and

(c) hybridizing a complementary single-stranded oligomeric compound to said identified single-stranded oligomeric compound, yielding a double-stranded oligomeric compound as the selected double-stranded oligomeric compound.

52. A method of identifying one or more optimized double-stranded oligomeric compounds comprising:

(a) cloning one or more target regions from a target RNA into a vector/plasmid construct;

(b) transfecting said vector/plasmid into a cell;

(c) contacting a cell transfected with said vector/plasmid with one or more double-stranded oligomeric compounds, said compounds having one strand hybridizable to said target region; and,

(d) identifying one or more double-stranded oligomeric compounds which inhibit target RNA levels by at least 50%.

53. An oligomeric compound, 8-80 nucleobases in length, targeted to a target RNA, wherein said oligomeric compound specifically hybridizes said target RNA and wherein said oligomeric compound inhibits RNA levels by at least 50% in both single-stranded and double-stranded forms.

54. The oligomeric compound of claim 53 wherein the oligomeric compound comprises one or more hairpin regions.

55. The oligomeric compound of claim 53 wherein RNA levels are measured in A549 cells.

56. An oligomeric compound, 8-80 nucleobases in length targeted to a target RNA, wherein said oligomeric compound has a least 80% sequence homology to the complement

of said target RNA and wherein said oligomeric compound inhibits RNA levels by at least 60% in both single-stranded and double-stranded forms.

57. The oligomeric compound of claim 56 wherein the sequence homology is at least 90%.

58. The oligomeric compound of claim 56 wherein the oligomeric compound has at least 2 mismatches as compared to the complement of the target RNA.

59. The oligomeric compound of claim 58 wherein the mismatches are internal or external base mismatches.

60. The oligomeric compound of claim 56 wherein no more than two of the four 3'-most nucleotides of the oligomeric compound are mismatches.

61. The oligomeric compound of claim 56 wherein said oligomeric compound has an IC_{50} no greater than 100nM.

62. The oligomeric compound of claim 56 wherein said oligomeric compound has an IC_{50} no greater than 10nM.

63. The oligomeric compound of claim 56 wherein said oligomeric compound is targeted to the 3'UTR, the 5'UTR, an intron:exon boundary, an exon:exon boundary, a start region or a coding region of the RNA.

64. The oligomeric compound of claim 56 wherein said oligomeric compound is targeted to the 3'UTR.

65. The oligomeric compound of claim 56 wherein said oligomeric compound is targeted to the 5'UTR.

66. The oligomeric compound of claim 56 wherein said oligomeric compound is targeted to an intronic portion of the RNA.

67. The oligomeric compound of claim 56 wherein said oligomeric compound is targeted to an exon.

68. The oligomeric compound of claim 56 wherein said oligomeric compound is targeted to an intron/exon boundary.

69. The oligomeric compound of claim 56 wherein said oligomeric compound has alternating linkages.

70. The oligomeric compound of claim 56 wherein the oligomeric compound has alternating modifications.

71. The oligomeric compound of claim 56 wherein every second nucleotide in the antisense strand of the double stranded oligomeric compound is modified.

72. The oligomeric compound of claim 71 wherein the first modified nucleotide is the 5'-most nucleotide of the oligomeric compound.

73. The oligomeric compound of claim 71 wherein the modifications are 2' modifications.

74. The oligomeric compound of claim 71 wherein the modifications are one or more of 2'-O alkyl, 2'-O-methoxyethyl, 2'-methoxyethoxy, 2'-dimethylaminooxyethoxy, 2'-dimethylaminoethoxyethoxy, 2'-methoxy, 2'-aminopropoxy, 2'-allyl, 2'-O-allyl (2'-O-CH_2-$CH=CH_2$), or 2'-fluoro.

75. The oligomeric compound of claim 56 wherein said oligomeric compound comprises:

a first segment;

a second segment; and,

a third segment comprising three or four nucleobases, said third portion located between said first and second segments;

wherein said first and second segments each have at least one modified nucleobase.

76. The oligomeric compound of claim 75 wherein said third segment has no modified nucleobases.

77. The oligomeric compound of claim 75 wherein said first and second segments each comprise at least one modified linkage/modification.

78. The oligomeric compound of claim 77 wherein said third segment has no modified linkages or modifications.

79. The oligomeric compound of claim 56 wherein said oligomeric compound hybridizes to at least a portion of the 3' UTR of said target RNA.

80. The oligomeric compound of claim 56 wherein said oligomeric compound comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside; said modified nucleoside adapted to modulate at least one of; binding affinity or binding specificity of said oligomeric compound.

81. The oligomeric compound of claim 56 wherein said oligomeric compound comprises at least seven 2'-O-methyl substitutions at the 3'-terminus of the oligomeric compound.

82. An oligomeric compound of claim 53 wherein the oligomeric compound has at least six mismatches as compared to the complement of the target RNA.